Novel butynyl group incorporated pyrrolopyrimidine analogues as potent dipeptidyl peptidase IV inhibitors

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ABSTRACT

A new chemical class of potent DPP-IV inhibitors has been structurally derived from our recently disclosed pyrrolopyrimidine scaffold by replacing cyanobenzyl with butynyl group. Systematic variations and structure-activity relationship studies have been conducted on the starting hit **51** (IC_{50}= 0.46 μM). Consequently, compound **78** (IC_{50}= 1.55 nM) was identified to be a potent, selective, and orally available lead, worth further evaluations and optimizations.

Keywords: DPP-IV inhibitor; type 2 diabetes; hit to lead optimization, SAR study.

1. Introduction

Type 2 diabetes (T2D) formerly referred to as non-insulin-dependent or adult-onset diabetes, results from the body's ineffective use of insulin and comprises over 90% of diabetes patients. With more than 220 million people affected worldwide, diabetes has emerged as an epidemic, reflecting the insufficient glycemic control and the urgency of more treatments [1]. Dipeptidyl peptidase IV (DPP-IV) inhibitors have been demonstrated to be effective and safe interventions that control blood glucose for good patient compliance and reduced risks of hypoglycemia and less side effects [2-4]. To date, Sitagliptin **1** [5-6], Vildaglipin **2** [7-9], Saxagliptin **3** [10], Alogliptin **4** [11] and Linagliptin **5** [12] are on the market worldwide (Figure 1). Among them, Linagliptin has demonstrated to be with superior potency and longest duration of action.

We previously reported a novel series of DPP-IV inhibitors bearing pyrrolopyrimidine scaffold, represented by compound **6** (Figure 2), which is currently undergoing systematic preclinical evaluations [13]. Inspired by Linagliptin, we replaced cyanobenzyl group with butynyl group on our published scaffold and led to compound **7**. However this replacement sharply decreased DPP-IV inhibitory ability with IC_{50} of 0.86 μM. Whereas none substituted compound **51** displayed much stronger inhibitory ability (IC_{50}= 0.46 μM, Figure 2). Systematic hit optimization has conducted mainly on the N-5 variations to led compound **78** (IC_{50}= 1.55 nM, Table 2). Preliminary evaluations demonstrated that **78** is a potent, selective, safe, and oral bioavailable lead.

Herein, we report the hit to lead optimization on the novel butynyl incorporated pyrrolopyrimidine scaffold and the evaluations of **78**.

2. Chemistry

The synthesis of compounds **21a**–**d** is outlined in Scheme 1. Briefly, 6-Methyluracil **11** was easily transformed to **12** via a typical nitrification. Subsequently, **13** was obtained via a condensation reaction of **12** and N, N-dimethylformamide dimethyl acetal, then **13** was treated by zinc powder in acetic acid

3. Results and Discussion

3.1. Novel butynyl group incorporated pyrrolopyrimidine scaffold.

According to the previous structure-activity relationship of xanthines [12], we noticed an increase in activity from N-7 aromatic group to N-7 aliphalic substitution. It suggests that aliphalic chain maybe more suitable for the hydrophobic S1 pocket of DPP-IV enzyme. Besides, N-7 butynyl derivatized scaffold has proved to be an effective measure to abolish side effects like hERG and M1 receptor inhibition in Linagliptin [12]. Thus we replaced the N-3 cyanobenzyl group of former pyrrolopyrimidine scaffold with butynyl group and generated the initial compound **7** (Table 1). Opposite to our expectation, this alternation sharply decreased the activity. In our earlier research, we noticed that bromo had little effect on the activity [13]. Thus none substituted compound **51** was generated and determined to have a much improvement in activity (Table 1). In vitro dipeptidyl peptidase (DPP) inhibitory IC_{50} values indicated that it is a moderate inhibitor against DPP-IV (0.46 μM) and free from inhibition of DPP-8, 9 (both >10 μM). It also did not inhibit the human Ether-à-go-go Related Gene (hERG) ion channel (IC_{50}> 100 μM), which may be related with cardiovascular risk [14-15]. Thus **51** is a potential and safe hit for further optimization.

*3.2. Optimization and structure-activity relationship study on hit compound **51**.*

The active sites and important residues of DPP-IV are well reported. And sufficient proves show the necessity of aminopiperidine substituent for xanthine derived scaffold. The whole fraction occupies the S2 subsite. Primary amine interacts with Glu205, Glu206, and Tyr662 through charge-reinforced hydrogen bonds [11-12]. Thus we turn on our attention to N-5 substituent and conduct the optimization mainly on the R_5 variations (Table 1).

Methyl substituted **52** sharply decreased the IC_{50} value by more than a half. It showed a positive signal for N-5 optimization. Incorporation with acetonitrile and butynyl group (**53** and **54** respectively) went on validating the increase in activity. Yet, 4-fluorobenzyl substituted **55** gave an exception for an extreme weak inhibition. However, the following ring(s) substitution showed stronger inhibition. Single

ring, 2-methylpyrimidinyl substituted **56** had one fold increase compared to aliphalic chain substitution **54**. Bicyclo rings (**57-60**) substitutions were obviously more effective to strengthen the DPP-IV affinity represented by **60** (IC_{50}= 7.05 nM). Generally on the N-5 variations from **51** to **60**, DPP-IV inhibitory activity is dependent on the size and direction of fraction to some extent.考虑加一句

Encouraged by 2-methylquinolinyl added **60**, **70-75** were synthesized to evaluated substituents' impact on quinoline (Table 2). Overall, halogen or methoxy incorporation obstructed the interaction between inhibitor and enzyme, exhibited by a small to moderate decrease in activity (**70-73**). Thus the smaller the atom is, the better the activity is. Besides electron effect had little effect on activity (**73**). Yet, it is worth to indicate that 7-quinoline substitution, especially small atom, may help the combination (**74-75**).

Simply switch from quinoline to qinazoline or quinoxaline fraction led to compounds **76-78** (Table 2). Compound **78** showed a very potent activity with 1.55 nM IC_{50}, as well as **76** with 5.93 nM IC_{50}. It suggested that hydrophobic and aromatic rings are more suitable to be placed at N-5. The corresponding interaction sites of the DPP-IV enzyme are probably hydrophobic at this position and have aromatic residues to interact with inhibitors by π-stacking. Also this may explain that halogen substitutes did not help to the affinity.

*3.3. Evaluations on compound **78**.*

Further inhibitory assays had conducted on the most potent compound **78**. Gratefully it did not inhibit hERG channel within 100 μM, and free from inhibition against DPP-8, 9 to 10 μM.

Pharmacokinetic profile of **78** in rat is in Table 3. Compared with our reported compound **6**, it has a longer half life in rat and twice the bioavailability, which is compatible with the marketed DPP-IV inhibitor (Alogliptin: 45%, Linagliptin: 50.7%) [12, 16]. Thus **78** is a promising lead worth of in depth evaluations and further optimization.

4. Conclusions

A general hit to lead optimization and SAR study have conducted on a new chemical class **51** (IC_{50}=

0.46 μM). N-5 variations indicate that hydrophobic and aromatic rings probably have stronger interaction with the DPP-IV enzyme at this position for the new class. As a result, compound **78** (IC_{50}= 0.46 μM) has generated and identified to be a promising lead compound with good selectivity, low cardiovascular risk, and compatible pharmacokinetic profile. Evidences show **78** is worth in depth evaluations and further optimizations will be carried subsequently.

5. Experimental Section

5.1. Chemistry.

All commercially available compounds and solvents were reagent grade and were used without further treatment unless otherwise noted. Reactions were monitored by TLC using Qing Dao Hai Yang GF_{254} silica gel plates (5 x 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) and by spraying with an ethanol solution of 2,4-DNP or Ninhydrin or by being fumed with iodine steam. Silica gel column chromatography was performed on silica gel (200–300 mesh) from Qing Dao Hai Yang. NMR spectra were recorded on a Bruker NMR AVANCE 400 (400 MHz) or a Bruker NMR AVANCE 500 (500 MHz). Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). MS data were measured on an Agilent MSD-1200 ESI-MS system.

*5.1.1. (R)-2-((2-(3-aminopiperidin-1-yl)-4-oxo-4,7-dihydro-3H-pyrrolo[2,3-d]pyrimidin-3-yl)methyl) benzonitrile (**21a**).*

A mixture of **20a** (13.1 g, 43.4 mmol), 3-(*R*)-aminopiperidine dihydrochloride (11.5 g, 66.0 mmol) and $NaHCO_3$ (17.4 g, 173.6 mmol) in a sealed tube containing 300 mL of ethanol was heated at 150 ℃ for 6 hours. The reaction mixture was subsequently cooled to room temperature and filtered. The resulting filtrate was concentrated in vacuo and then purified by flash chromatography to yield compound **21a** as white powder. Yield: 67.3%. ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 7.64 (1H, d, *J*= 6.8 Hz), 7.45 (1H, t, *J*= 7.6 Hz), 7.31 (1H, t, *J*= 7.2 Hz), 7.07 (1H, d, *J*= 8.0 Hz), 6.81 (1H, d, *J*= 3.2 Hz),

6.64 (1H, d, *J*= 3.2 Hz), 5.55 (2H, AB q, *J*= 38.8 Hz, 16.0 Hz), 3.21-3.17 (1H, m), 3.05-3.02 (2H, m), 2.82-2.77 (2H, m), 1.94-1.90 (1H, m), 1.73-1.68 (1H, m), 1.63-1.55 (1H, m), 1.45-1.43 (1H, m). ^{13}C-NMR (100 MHz, MeOD) δ ppm: 22.83 (1C), 31.49 (1C), 45.41 (1C), 47.11 (1C), 51.34 (1C), 56.96 (1C), 102.17 (1C), 104.02 (1C), 110.46 (1C), 116.98 (1C), 120.19 (1C), 126.69 (1C), 127.47 (1C), 132.72 (1C), 133.01 (1C), 141.66 (1C), 147.45 (1C), 155.75 (1C), 161.00 (1C). ESI-MS calculated for ($C_{19}H_{20}N_6O$) $[M+H]^+$, 349.2, found 349.1.

5.2. In vitro inhibition of DPP-IV, DPP-8 and DPP-9.

Solutions of test compounds in varying concentrations（≤10 mM, final concentration) were prepared in dimethyl sulfoxide (DMSO) and were diluted into assay buffer made from the following components: 20 mM Tris (pH 7.4); 20 mM KCl; and 0.1mg/mL BSA. Human DPP-IV (0.1 nM, final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated with the addition of Gly-Pro-AMC (H-glycyl-prolyl-7-amino-4-methylcoumarin; Sigma-Aldrich; 10 μM, final concentraton). The total volume of the reaction mixture was 100 μL. The kinetics of the reaction was monitored (excitation at 400 nm; emission at 505 nm) for 5-10 minutes, or an endpoint was measured after 10 minutes. Inhibition constants (IC_{50}) were calculated from enzyme progress curves using standard mathematical models.

5.3. In vivo pharmacokinetic study.

Adult male SD rats (n= 4/group) were administrated with the test compounds dissolved in distilled water at a single dose of 20 mg/kg or 25 mg/kg for oral administration and 5 mg/mL in vein. Blood samples of 100-200 μL were collected from the orbit at 11 time points within 24 hours. The blood concentration of test compounds was determined by LC-MS/MS. The PK parameters were obtained from the pharmacokinetic software DAS. 2.0.

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